Separate Sex-Influenced and Genetic Components in Spontaneously Hypertensive Rat Hypertension

Monte E. Turner, Mark L. Johnson, and Daniel L. Ely

Previous results from our laboratory indicated two major genetic components of spontaneously hypertensive rat (SHR) hypertension, an autosomal component and a Y chromosome component. Two new substrains, SHR/a and SHR/y, were developed using a series of backcrosses to isolate each of these components. The SHR/a substrain has the autosomal loci and X chromosome from the SHR strain and the Y chromosome from the Wistar-Kyoto (WKY) rat strain. The SHR/y substrain has only the Y chromosome from the SHR and autosomal loci and X chromosome from the WKY strain. Throughout these breeding programs parents were chosen at random without selection for blood pressure. Males of both substrains maintained blood pressures over 180 mm Hg. Comparisons of blood pressure in these new substrains with the original parental strains can be used to determine the relative proportions of each genetic component in hypertension. The Y chromosome component contributes 34 mm Hg, which is the difference between SHR/y male and WKY male blood pressure. The total autosomal component contributes 46 mm Hg, which is the difference between SHR/a male and WKY male blood pressure. The autosomal component is a sex-influenced trait; males in the SHR/a strain have significantly higher pressures than SHR/a females. Of the 46 mm Hg estimated for the autosomal component, 41 mm Hg is the result of these loci interacting with male phenotypic sex. This sex-influenced component is separate and distinct from the Y chromosome component. (Hypertension 1991;17:1097-1103)

In the spontaneously hypertensive rat (SHR), a well-studied animal model of human essential hypertension, blood pressures of males are significantly elevated above those of females. In dissecting the genetic basis of SHR hypertension, the origin of this sex difference has not been explained. A number of genetic traits either are associated with or differ with phenotypic sex. There are at least three different genetic mechanisms to account for these types of traits. A sex-linked trait is the result of a gene that is located on the X chromosome. A holandric trait is the result of a gene on the Y chromosome. These two types of traits show associations with sex in mammalian systems because females have two X chromosomes and males have one X and one Y chromosome. A third trait, sex-influenced, is also affected by phenotypic sex. For such a trait, a male and female with the same genotype at the sex-influenced locus may have different phenotypes. An example of such a trait in human pedigrees is male pattern baldness. The gene for this trait acts as an autosomal dominant in males but as an autosomal recessive in females. In a simple sense, a sex-influenced trait is a gene whose phenotype is modified by the environment, except that the environment in this case is phenotypic sex. The genetic locus for a sex-influenced trait can be found anywhere in the genome, and sex-linked or holandric traits have no greater chance of being sex-influenced than autosomal traits.

Genetic crosses between Wistar-Kyoto rats (WKY) and SHR have been used to elucidate the genetic basis of this hypertension. Surprisingly, we have found that the blood pressure of hybrid offspring was dependent on the strain of the father. Male offspring with an SHR father had significantly higher pressures than sons with a WKY father. These crosses suggested that there are two major components of SHR hypertension: one component is a genetic locus on the SHR Y chromosome, a holandric trait, and the second component is an autosomal locus or loci. Finding a Y chromosome locus is also consistent with the observation that SHR males have higher blood pressures than SHR females since
males would have both the Y and autosome components, while females would only have the autosomal component. However, this is only a portion of the picture because in those crosses with a WKY father, sons still had significantly higher blood pressures than daughters.\(^4\) The hypertensive Y chromosome was not present in these males, so if only the Y chromosome were responsible for the sex differences, these sons and their sisters should have the same blood pressures. This result would indicate that the autosomal component may be a sex-influenced trait. In some way the environment of maleness or femaleness modifies the hypertensive effects of the SHR autosomal genes.

The origin of the SHR strain is instructive in understanding the results described above. The SHR strain was originally derived through the selective breeding of WKY males and females with increased blood pressure.\(^5\) There was an almost immediate response to this selection, with almost 100% hypertension by generation three.\(^6\) This type of selective response would be possible if only a few genetic loci were involved. It is also important that in the original selected parents, males had higher pressures than females. These original observations and the rapid response to selection are consistent with the finding of a Y chromosome component and the possibility of a sex-influenced genetic component.

The purpose of this study is to describe the results of experiments designed to quantitate the individual hypertensive effects of two genetic components. The autosomal component was tested for the influence of phenotypic sex on these loci. A description of two new backcross substrains of SHR that separate the two genetic components of SHR hypertension is included.

**Methods**

The original SHR and WKY strains were obtained from Harlan Sprague Dawley (Indianapolis, Ind.), which obtained the initial SHR breeding stock from the National Institutes of Health. According to the most current Harlan Sprague Dawley genetic monitoring report, this strain is comparable with the most genetically authentic hypertensive strains in the United States that were derived from the National Institutes of Health colonies (personal communication, 1988).

Two new substrains were developed to separate the Y chromosome and the autosomal loci, which are the two genetic components of SHR hypertension. The first substrain, designated SHR/y, contains an SHR Y chromosome and autosome genes of WKY origin. A WKY female was crossed with an SHR male and F\(_1\) sons were selected. These F\(_1\) sons were crossed with a WKY female, and F\(_2\) sons were selected. This mating scheme was continued each generation with sons crossed with WKY females. All sons selected for breeding were picked at random without regard for blood pressure. The final blood pressures presented in this report are from generation F\(_7\) (Table 1). At this generation, the autosomal loci should be over 99% WKY with any remaining SHR loci being a random mix and not necessarily those loci affecting blood pressure. Since sons were selected each generation, the Y chromosome is of SHR origin, although the pseudoautosomal region could contain loci of WKY origin.

The second substrain, designated SHR/a, contains autosomal loci of SHR origin and a WKY Y chromosome. An SHR female was crossed with a WKY male, and F\(_1\) sons were selected. These sons were crossed with an SHR female, and F\(_2\) sons were selected. Sons were selected from each generation without regard for blood pressure and crossed with an SHR female. The F\(_3\) rats used in these experiments contain on average over 99% SHR loci (Table 1) with a WKY Y chromosome. Although we have designated this strain SHR/a and refer to the autosomal component, this substrain also contains the SHR X chromosome, and any effect of this chromosome would be included in the autosomal effects.

Animals were maintained at room temperature (26–28°C) with a 12-hour light/dark cycle (6 AM–6 PM, light/6 PM–6 AM, dark), and were provided with water and Purina lab chow ad libitum. A typical breeding box (40×50×20 cm) consisted of three females and one male housed in aspen shavings (changed once per week; American Excelsior, Cleveland, Ohio). All animals were maintained and treated according to National Institutes of Health guidelines.

Blood pressures were measured by tail sphygmomanometry (Narco Biosystems, Houston, Tex.).\(^7\) Blood pressures were measured for the following strains, substrains, and generations: SHR/a F\(_3\), 14–24 weeks of age, n=3 males; SHR/a F\(_2\), 14–24 weeks of

<table>
<thead>
<tr>
<th>Substrain</th>
<th>Generation</th>
<th>% SHR genes</th>
<th>% WKY genes</th>
<th>Y chromosome</th>
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<td>50.0</td>
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</tr>
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<td>3</td>
<td>87.5</td>
<td>12.5</td>
<td>WKY</td>
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<tr>
<td></td>
<td>4</td>
<td>93.8</td>
<td>6.2</td>
<td>WKY</td>
</tr>
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<td>1.6</td>
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<td>8</td>
<td>99.6</td>
<td>0.4</td>
<td>WKY</td>
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<td>SHR</td>
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<td></td>
<td>8</td>
<td>0.4</td>
<td>99.6</td>
<td>SHR</td>
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SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto rat.
Sex-Influenced Hypertension

Figure 1. Plot of average male blood pressure of backcross generations with respect to age for the SHR/y substrain. Original parental strains, SHR and WKY, are included for comparisons. F1 data are reproduced from Ely and Turner. SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto rat; F1, generation 1; F4, generation 4; F5, generation 5; F7, generation 7; SHR/y, rat substrain containing an SHR Y chromosome and autosomal genes of WKY origin.

Results

Blood pressures in males during the development of the new substrains, SHR/a and SHR/y, are indicated in Figures 1 and 2. In the SHR/y development (Figure 1), there were no consistent significant differences between age and generations, but pressures were significantly lower than SHR and higher than WKY (p<0.01). In the SHR/a strain (Figure 2), F7 pressures were significantly higher than the F1 generation. Generation F7 pressures in SHR/a were significantly lower than SHR and higher than WKY (p<0.01).

Blood pressures in females during the development of the SHR/a substrain are shown along with the two original parental blood pressure profiles in Figure 3. SHR female pressures were elevated above WKY females, and generation F7 SHR/a females were significantly higher than WKY females at 18 weeks of age but not significantly different than SHR females. Comparison with Figure 2 demonstrates the overall lowering of blood pressure in females versus males in the SHR/a substrain.

Table 2 lists average blood pressures for males of each substrain, parental strains, female pressures for the SHR/a substrain, and the parental strains. These are representative pressures taken at about 20 weeks of age and were used to calculate the relative proportions of each component. Within this group, male pressures of the SHR/a and SHR/y substrains were significantly lower than SHR (p<0.01) and significantly higher than WKY males (p<0.01). The male pressures of the two substrains were not significantly...
different. Female pressures of the SHR/a substrain were significantly higher than WKY females \((p<0.05)\) but not different from SHR. Male pressures in the SHR/a, SHR, and WKY strains were significantly higher than female pressures (SHR/a and SHR, \(p<0.01\); WKY, \(p<0.05\)).

**Discussion**

Many studies have compared physiological and molecular differences between the SHR and WKY strains.\(^8\)-\(^{12}\) The goal of those research efforts was to relate observed differences to the development of hypertension in the SHR model. Since there are two separate genetic components involved in SHR hypertension,\(^4\) any difference or response may be an effect of either component, both components, or neither component. It is necessary to separate the components to study each alone without the possibility of interfering effects of the other. Using a method of continuous backcrossing of sons to the maternal strain allows the replacement of the autosomal background and the X chromosome.\(^13\) Each generation of backcrossing reduces the paternal autosomal component by 50% (Table 1). By generation \(F_6\), the new backcross substrains have over 99% of the maternal autosomal genes with the paternal Y chromosome (Table 1). Through this method we have expanded the SHR rat model to include the hypertensive SHR strain, the normotensive WKY strain, and two genetic substrains, SHR/a and SHR/y, each having different hypertensive genetic components. In each substrain it is unclear whether a single locus or multiple loci are involved in raising blood pressure.

**SHR/a Substrain**

Blood pressures in this substrain have increased since the original \(F_1\) generation (Figure 2). This result was expected because crosses by Tanase et al.\(^{14}\)-\(^{16}\) had indicated the autosomal loci acted in an additive manner. Blood pressure increased as the strain progressed through backcrossing from being heterozygous for the hypertensive loci in generation \(F_1\) to being essentially homozygous for these loci by generation \(F_7\). This strain can be used as a borderline purebred hypertensive strain. Blood pressures in this
substrain were significantly lower than the parental SHR strain (Table 2).

**SHR/y Substrain**

The blood pressures taken during the development of this substrain have very little variation from generation to generation (Figure 1). At generation F1, all autosomal loci affecting blood pressure were heterozygous. At generation F7, these loci should be homozygous for the normotensive alleles. Since there is no significant difference in blood pressures in the SHR/y substrain from generation F1 to F7, the SHR autosomal alleles act as recessives in the presence of an SHR Y chromosome. Like the SHR/a substrain, the SHR/y is a borderline hypertensive strain, but the genetic basis of this hypertension is separate and most likely different from the SHR/a substrain.

**Y Chromosome Effect**

Our previous results demonstrated the hypertensive effect of the SHR Y chromosome,4 which led to the development of a substrain with only the SHR Y chromosome. The hypertensive effect of the SHR Y chromosome is confirmed in the SHR/y substrain compared with the WKY strain (Figure 1, Table 2). The SHR/y males had significantly higher blood pressures than WKY males, and significantly lower blood pressures than the SHR males. This substrain

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**TABLE 2. Average Blood Pressures From Generation 7 of the Two Backcross Substrains, SHR/a and SHR/y, and the Two Parental Strains, Spontaneously Hypertensive Rats and Wistar-Kyoto Rats**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>n</th>
<th>Average ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR/a</td>
<td>Male</td>
<td>14</td>
<td>194±8*†</td>
</tr>
<tr>
<td>SHR/a</td>
<td>Female</td>
<td>6</td>
<td>153±6§</td>
</tr>
<tr>
<td>SHR/y</td>
<td>Male</td>
<td>14</td>
<td>182±4*†</td>
</tr>
<tr>
<td>SHR</td>
<td>Male</td>
<td>12</td>
<td>230±7†</td>
</tr>
<tr>
<td>SHR</td>
<td>Female</td>
<td>8</td>
<td>147±2§</td>
</tr>
<tr>
<td>WKY</td>
<td>Male</td>
<td>8</td>
<td>148±4§</td>
</tr>
<tr>
<td>WKY</td>
<td>Female</td>
<td>8</td>
<td>132±5</td>
</tr>
</tbody>
</table>

SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto rat.

* p<0.01 compared with SHR male
† p<0.01 compared with WKY male.
§ p<0.01 compared with SHR/a male
& p<0.05 compared with WKY female.
carries only the SHR Y chromosome in a WKY autosomal and X chromosome background. Any increase in blood pressure over WKY males must be a direct result of the hypertensive effect of the SHR Y chromosome. The relative effect of the SHR Y chromosome can be determined from the difference between the average blood pressure of the SHR/y males and the average blood pressure of WKY males (Table 2), which in these experiments was 34 mm Hg.

**Autosomal Effect**

The Y chromosome was not the only genetic component in the development of SHR hypertension; an autosomal genetic component is also involved. The SHR/a strain contains only the autosomal loci from the original SHR parental strain. Males from the SHR/a strain carry the WKY Y chromosome. Male blood pressures in this strain were significantly higher than that of WKY males and SHR/a females, and it was significantly lower than that of SHR males (Table 2). This component can be determined from the difference between the average blood pressure of SHR/a males and the average blood pressure of WKY males (Table 2), which was 46 mm Hg.

Females in the SHR/a strain should be genetically identical to SHR females because both have autosomes and X chromosomes of SHR origin. As such, the blood pressures of these two female groups should not differ significantly, and in this study they did not (Table 3). Both SHR/a and SHR female blood pressures were significantly higher than those of WKY females.

**Sex Influence**

In testing the hypothesis that the autosomal genes are a sex-influenced trait, blood pressures of SHR/a females were compared with those from SHR/a males. Both of these groups have SHR autosomes and X chromosomes of SHR origin. As such, the blood pressures of these two female groups should not differ significantly, and in this study they did not (Table 3). Both SHR/a and SHR female blood pressures were significantly higher than those of WKY females.

**Relation of Y and Autosomal Components**

If the two components, Y chromosome and autosomes, were strictly additive in their interaction, the sum of the SHR/a and SHR/y increases, 80 mm Hg (46+34 mm Hg), should equal the SHR male blood pressure minus the WKY male blood pressure, 82 mm Hg (230-148 mm Hg). This additive effect only occurs when the hypertensive loci are homozygous. This conclusion results from the development of the SHR/y strain, which did not have significant decreases in pressure from generation F1 to generation F3 (Figure 1). Our data would indicate the two components are additive. Previous studies with crosses between WKY and SHR have concluded that the genes responsible for the SHR hypertension acted in an additive manner. However, these previous studies only indicated the possible relation of the autosomal loci to each other and not the interaction of the autosomal and Y chromosome components.

It is possible that the autosomal and Y chromosome components are related in their mechanisms rather than totally independent. The Y chromosome gene may also be influenced to a positive degree by male phenotypic sex. In our results, the WKY male blood pressures were significantly elevated over those of the WKY females; this could be an indication of a sex-influenced effect on the normotensive alleles or a small effect of the WKY Y chromosome or both. Whether the Y chromosome gene is sex-influenced, as is the autosomal component, cannot be tested until the gene itself is isolated.

In conclusion, the development of the SHR/a and SHR/y substrains illustrates that the two major components of SHR hypertension can increase and maintain increased blood pressure independently of each other. The SHR/a strain maintains male blood pressures above 190 mm Hg and SHR/y above 180 mm Hg. Comparison of SHR/a males and females indicates the autosomal loci have a sex-influenced
component that augments the blood pressure rise in males above those in females. The blood pressure increase of SHR males over WKY males was due to autosomal loci, sex-influence of these autosomal loci, and the SHR Y chromosome. These components apparently interact in an additive fashion.

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